

RESEARCH HIGHLIGHT

Erythrocytes serve as a template to create nanozymes with uniform Fe single-atom sites for healing bacteria-infected wound

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Bacterial infection is recognized as one of the biggest global health threats, causing millions of people's death every year (Wang et al., 2020). Traditional antibiotics-based therapies have been widely utilized to control this issue (Kohanski et al., 2010); however, their excessive use leads to the emergence of antibiotic-resistant bacteria, endangering to human health (Levy and Marshall, 2004). Hence, another antibacterial strategy free from antibiotics and based on different bactericidal mechanisms, is necessary to effectively resolve the current problematic situation.

In this regard, nanomaterials with enzyme-like properties (nanozymes) have gathered much attraction due to their capability to kill bacteria by utilizing their unique enzyme catalysis-based mechanisms (Mei et al., 2021). Among various nanozymes developed so far, Fe-based ones have been most widely studied and used as antimicrobial agents, based on their peroxidase-like activities to generate hydroxyl radicals from H_2O_2 , which attacks bacterial cell membranes and induce cell death. However, the catalytic efficiency of Fe-based nanozymes is often lower than that of natural counterpart enzymes (e.g., horseradish peroxidase; HRP). To overcome this limitation, many studies have been conducted, and nowadays, the nanozymes preserving Fe single-atom sites have gathered significant attention because they can exhibit the highest level of peroxidase-like activity, even higher than that of HRP. The Fe single-atom is usually coordinated with four N sites, which mimic the structure of heme within the active site of HRP, and it then efficiently facilitates electron transfer and peroxidase-like action from the increased atomic utilization efficiency (Guo

et al., 2024). Nevertheless, in addition to single-atom Fe, Fe clusters or Fe-Fe bonds are produced during the conventional synthesis of Fe single-atom nanozymes because the employment of external iron precursors with inevitable pyrolytic carbonization at high temperatures often yields a lower ratio of single-atom Fe but a higher ratio of Fe clusters (Xi et al., 2021). Recently, inspired by the natural composition of erythrocytes (red blood cells), which contain approximately 260 million hemoglobin molecules per cell and each hemoglobin contains four heme molecules with FeN_4 single-atom sites, Gao and his research team (Wang et al., 2024) developed a unique erythrocyte-templated strategy to synthesize peroxidase-like nanozymes that have uniform and abundant Fe single-atom sites without any meaningful Fe clusters (Figure 1).

Erythrocyte-templated Fe single-atom nanozyme (ETN) was prepared via sequential steps of cell fixation, porous salinization, and high-temperature pyrolytic carbonization. Each step is essential to maintain a rounded and biconcave cellular shape with honeycomb-like morphology, better aqueous dispersibility, and a relatively small size of the ETN. Through diverse characterization methods, the ETNs were demonstrated to fully preserve single-atom FeN_4 active sites, which were derived from the heme active centers and evenly distributed within the hemoglobin molecules throughout the erythrocytes. Importantly, the number of single-atom Fe-based active sites in one ETN was estimated to be $\sim 2.53 \times 10^8$, which is similar to the magnitude of hemoglobins in erythrocytes (2.60×10^8 per cell), indicating that the single-atom Fe in erythrocytes was efficiently retained in the ETN. Since the peroxidase-like activity

can be efficiently induced from the Fe single-atom sites, the ETNs exhibited a significant peroxidase-like activity, which is higher than that of previous Fe single-atom nanozymes that might contain single-atom Fe as well as Fe clusters. The authors also found that mouse-derived ETNs showed the highest peroxidase-like activity among the ETNs derived from other eight species, because the Fe content of the mouse-derived ETN was the highest. Interestingly, the nanozymes prepared from non-erythrocyte cells, such as Raw 264.7 and HaCaT, did not show any significant enzymatic activity, probably due to the absence of intrinsic Fe. These experimental observations (Wang et al., 2024) convincingly support that erythrocytes can be an exceptional template for synthesizing peroxidase-like Fe single-atom nanozymes.

Nanozyme-based antibacterial strategies have been conducted via three ways: (i) reactive oxygen species (ROS) generation; (ii) HOBr/Cl generation, and (iii) extracellular DNA clearance (Mei et al., 2021). The ETNs are also expected to have antibacterial activity due to their high peroxidase-like activity. Indeed, Gao's team (Wang et al., 2024) found that the ETNs could effectively kill a broad spectrum of bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA). This antibacterial activity was attributed to the synergistic effects of peroxidase-driven ROS generation and near-infrared (NIR) induced photothermal effect, which induced critical damages to bacterial cell membranes. The peroxidase-like action of ETNs also led to glutathione (GSH) depletion by the disruption of its intrinsic balance in intracellular environments, resulting in a rapid elimination of bacteria. Alteration in GSH

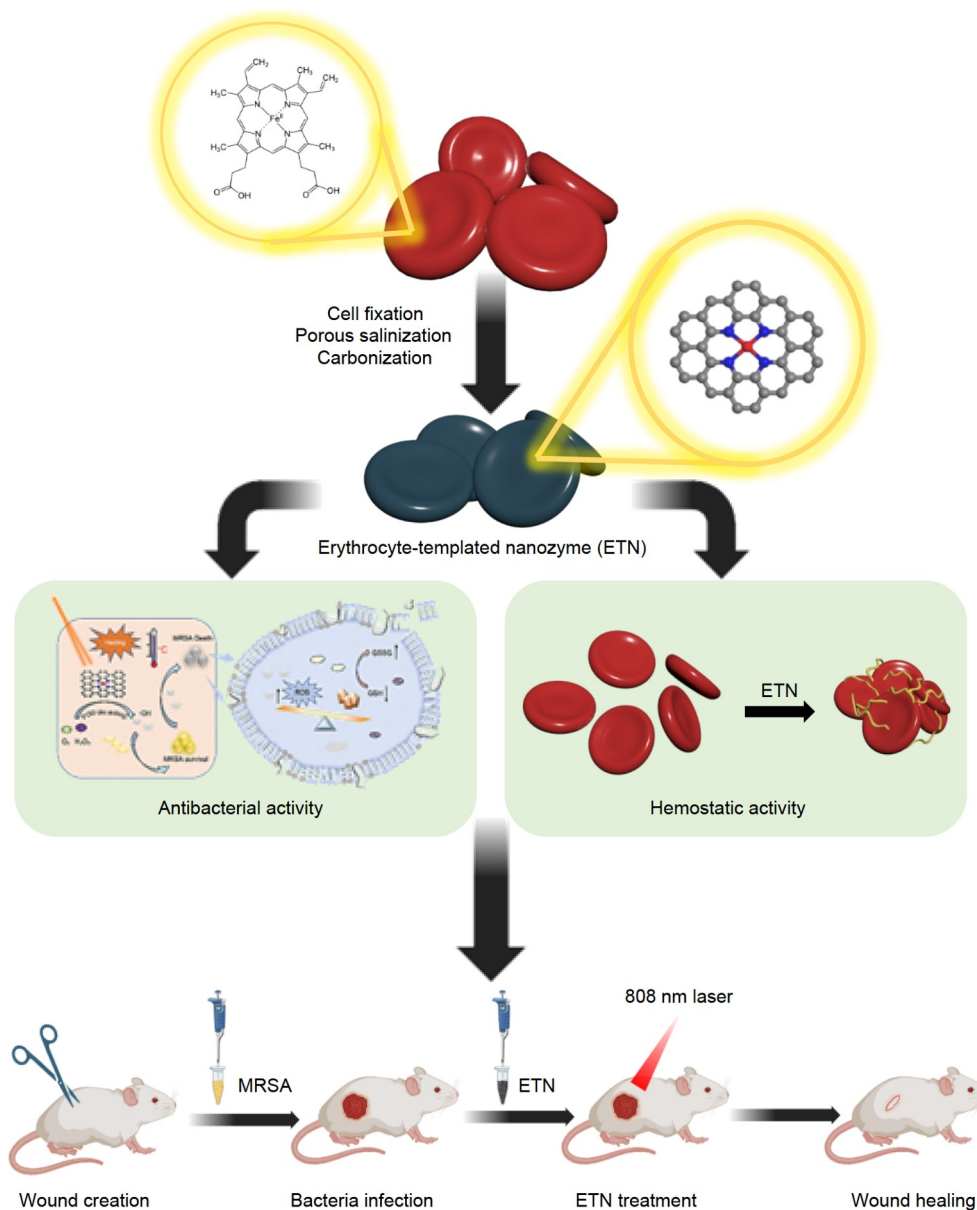


Figure 1. Schematic illustration of using erythrocytes to create Fe single-atom nanozyme (ETN) that has antibacterial and hemostatic activities for bacteria-infected wound healing.

content is believed to directly induce the ferroptosis-like death, which has been proven in many other antibacterial nanozymes (Xiao et al., 2022). Of note, the ETN-based antibacterial strategy is not specific to MRSA, and other Gram-positive like *Staphylococcus aureus* (*S. aureus*) and Gram-negative like *Escherichia coli* (*E. coli*) have also been effectively eradicated, confirming that the ETN has a broad antibacterial spectrum.

Another important finding by Gao's team is that the developed ETNs can participate in the intrinsic coagulation cascade pathway owing to their outstanding hemostatic property (Wang et

al., 2024), which is possibly achieved by their excellent adsorption property from the honeycomb-like structure consisting of 3D porous networks. With blood clotting tests, it was clearly demonstrated that the ETNs promoted blood clotting. Further analyses, such as Western blot and SEM analyses, confirmed that the ETNs directly adsorbed fibrinogen and prothrombin, which helps to produce blood clots, promote blood coagulation, and prevent uncontrolled bleeding, especially from non-compressible wounds.

Based on the efficient antibacterial and hemostatic properties of ETNs, Gao's team finally explored their potential for bacter-

ia-infected wound healing. For evaluating *in vivo* therapeutic effects, an injury-infection mouse model inoculated with sufficient numbers of MRSA was developed. The experiments clearly showed that ETN/H₂O₂/NIR-based strategy, which utilizes both peroxidase-like and photothermal effects, was highly effective in wound healing. Importantly, only a negligible number of MRSA was detected from the wounds after the application of ETN/H₂O₂/NIR-based strategy. The residual wound area was reduced by approximately 75%, and moreover, fibroblast proliferation and intact skin tissue structure were observed, indicating a promoted

recovery of bacteria-infected wounds. The weight and health condition of the mouse were nearly unchanged during the test, revealing the absence of adverse reactions from the treatment with the ETNs.

In conclusion, investigations by Gao and his coworkers (Wang et al., 2024) suggest that erythrocytes hold great promise as a template to create nanozymes preserving uniform and abundant Fe single-atom sites throughout the matrices of the ETN. ETNs are highly effective in healing bacteria-infected wound due to their peroxidase-like, photothermal, and hemostatic properties, which are achieved from their unique architectures. This study opens a new avenue toward the utilization of biomaterials as a template to create novel nanozymes with exceptional properties, which are highly beneficial in

achieving practical applications, especially in biomedical area.

Compliance and ethics

The author(s) declare that they have no conflict of interest.

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